Original Research Article

Level of Natural Coagulation Inhibitors among Pregnant Women in Southwest Nigeria

Abstract

Background: The risk of venous thromboembolic events (VTE) is high during pregnancy due to both physiologic changes in pregnancy and the impact of inherited and acquired thrombophilia. Protein S (PS), Protein C (PC) and Antithrombin III (ATIII) deficiencies have been found in some pregnant women with recurrent miscarriages and sudden death. This study aimed to determine the changes in the level of plasma Protein C, Protein S and Antithrombin III levels, its correlation with normal pregnancy.

Methods: The study was a comparative cross-sectional study conducted among seventy-five normal pregnant women who were selected using a simple random sampling technique with seventy-five age-matched healthy nonpregnant women. Blood samples were collected for analysis of Protein C, Protein S and Antithrombin III using the Enzyme-linked Immunosorbent assay method. A semi-structured questionnaire was used as the survey instrument and Statistical analysis of data was done using SPSS version 24.

Results: The mean ages of the respondents were 32.6±4.6 and 34.5±6.9 years for the subjects and controls respectively. Natural coagulation inhibitors(NCI) show a gradual decrease across the semesters of pregnancy. There was a statistical significance in the level of antithrombin III and protein S in the first trimester, $p<0.05$. when compared with the control of Protein S of 4.78 ± 0.65 ng/mL and Antithrombin III of 554.16 ± 54.65 ng/mL respectively.

Conclusion: It was demonstrated that there was an accompanying reduction of NCI across the trimester compared with the controls. Antithrombin III and Protein S have a significant relationship with the gestation periods. Antithrombin III decreased as pregnancy advanced while Protein S decreased significantly from the first trimester to the second trimester and was maintained at that level throughout the pregnancy.

Keywords: natural coagulation inhibitor, pregnancy, southwest, Nigeria,

Introduction

The coagulation system undergoes significant changes during pregnancy; an increase in levels of some of the clotting factors, decreased concentrations of some of the natural anticoagulants and diminishing fibrinolytic activity, thus producing a state of hypercoagulability.[1,2,3] This phenomenon, supposedly due to hormonal changes, helps in maintaining placental function during pregnancy, and protects from fatal hemorrhage during delivery.[4,8]

The risk of venous thromboembolism (VTE) is high during pregnancy due to both physiologic changes of pregnancy and the additional impact of the inherited and acquired thrombophilias.[2,5-6] The overall rate of venous thromboembolic events in pregnancy is 200 per 100,000 deliveries.[2,7-8]The main risk appears to occur in the postpartum period where the incidence increases about 3-fold and is estimated at 500 per 100,000.[2,7] These events are deep vein thrombosis as opposed to the more serious pulmonary embolism.[2,10]

Inherited and acquired thrombophilia contribute further to an increased predisposition to thrombotic events.[2,9] The overall impact of inherited and acquired thrombophilia is low in the non-pregnant population, and the majority of patients never experience a thrombotic event.[2,8,9-10] During pregnancy, however, the increased risk of thrombosis in patients with inherited and acquired thrombophilia can be substantial and warrants consideration, especially as thrombosis is one of the leading causes of mortality during pregnancy.[2,7,9]

Positive efforts have been made in reducing maternal death from postpartum hemorrhage (PPH) over years.[9,9] Other causes of maternal death are frequently undiagnosed or under-diagnosed.[7] Most of these deaths result from thrombo-embolic disease in pregnancy.[7,13,11]Approximately 50% of women with venous thrombo-embolic (VTE) disorders detected during pregnancy or puerperium have an underlying hereditary thrombophilia in addition to the acquired risk.[6] Several investigators have reported an association between thrombophilia and adverse pregnancy outcomes caused by uteroplacental thrombosis. Other groups, however, have failed to confirm this association.[7,8,16,12]

Protein S (PS) deficiency and protein C deficiency (PC) have been found in the general population with pregnant women not excluded and Antithrombin III (ATIII) deficiency is associated with recurrent miscarriages.[5,17,13]

Several reports have regarded coagulation profiles as markers of thromboembolic disorder in pregnancy.[10,14,15]A study done in Kano Nigeria showed that platelet count, and factor VIII concentrations are increased during pregnancy with significant reduction of protein C.[3] However, there is paucity of information on the level of natural coagulation inhibitors during pregnancy from this environment. This research work aimed at determining the level of natural coagulation inhibitors in pregnant women in the three trimesters of pregnancy.

Methodology

Study Area

This study was conducted at the ante-natal clinic, booking clinic and labor ward of Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Oyo State, Nigeria.

The hospital serves as a research and training institution for undergraduate and postgraduate medical programs as well as a major referral centre. The strategic location of the teaching hospital in Ogbomoso enables it to serve the people of Oke-Ogun, Oyo town and its environs, Ogbomoso and its environs, some parts of Osun as well as Kwara state with qualitative health care delivery.

Study Design

The study was a comparative cross-sectional hospital-based study.

Pregnant women between ages of 15- 49 years with serum or ultrasound confirmation of pregnancy were included in the study after obtaining informed consent.

Sample Size

The sample size for patients was calculated using the statistical formula comparative study.[16] The minimum sample size derived was 24.55. To enhance the power of this study, a total sample size of 75 was considered for both subjects and controls. Therefore, a total sample size of 150 was used. From this, 75 normal pregnant women were subdivided into three groups; 25 in their first trimester, 25 in their second trimester, 25 in their third trimester and 75 age-matched non-pregnant women as control.

Procedure

A semi-structured questionnaire was used as the study instrument to obtain information on socio-demographic characteristics and clinical history. After obtaining informed consent, 5mls of venous blood was collected from each subject into EDTA bottle. The specimens were centrifuged, the plasma was separated into aliquot bottles and stored at a temperature of -30^0C for analysis of Protein C, Protein S, Antithrombin III assays. The enzyme-linked immunosorbent assay (ELISA) method was used for the quantitative determination of Protein C, protein S Antigens and Human Antithrombin III.

Data Analysis

The statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 24. A multivariate analysis was done. Mean and standard deviation were used to report baseline continuous variable while frequency and proportions were used to report categorical variables. Pearson's chi-squared, student T- test were used to compare variables as appropriate.

Ethical Approval

Approval was obtained from the ethical committee of the LAUTECH Teaching Hospital (LTH) Ogbomosho, Oyo State Nigeria.

Results

One hundred and fifty (150) questionnaires were administered in all; seventy- five for subjects which were subdivided into twenty- five pregnant women each in their first trimester, second trimester and third trimester respectively (twenty- two of the subjects were primigravida) and seventy- five controls who were equally age matched healthy non-pregnant women.

Table 1 shows the demographic profile of the participants. The mean ages of the subjects and control group were 32.6±4.6 and 34.5±6.9 years respectively. The majority of participants in both groups were married (Control: 84%, Cases: 98.7%).

Table 1: Socio-demographic characteristics of subjects

Variables	Control group n (%)	Subjects n (%)	χ^2	p-value
Age (Years)				
20 - 24	7 (9.3)	9 (12.0)	5.287	0.508
25 - 29	23 (30.7)	26 (34.7)		
30 - 34	34 (45.3)	30 (40.0)		
35 – 39	6 (8.0)	9 (12.0)		
40 – 44	1 (1.3)	1 (1.3)		
45 – 49	4 (5.4)	0 (0.0)		
Mean ± SD	34.5 ± 6.9	32.6 ± 4.6		
Religion				
Christianity	62 (82.7)	53 (70.7)	3.091	0.082
Muslim	13 (17.3)	22 (29.3)		
Marital Status				
Married	63 (84.0)	74 (98.7)	10.247	*0.006
Single	10 (13.3)	1 (1.3)		
Widowed	2 (2.7)	0 (0.0)		
Ethnicity				
Yoruba	75 (100)	70 (93.3)	5.172	0.075
Igbo	0 (0.0)	2 (2.7)		
Others	0 (0.0)	3 (4.0)		
Level of education				
Primary education	0 (0.0)	1 (1.3)	3.934	0.140
Secondary education	4 (5.3)	10 (13.3)		
Tertiary education	71 (94.7)	64 (85.3)		

χ^2: Chi square statistic, p-value < 0.05 indicates significance

Table 2 shows that m the majority of participants in the subjects and control group have had a history of two to three pregnancies before (study: 56%, control: 58.7%) respectively and 37.7% of the subjects and 34.5% of the control group had previous miscarriages respectively. Two (3.8%) of the subjects and none of the control group had a history of intrauterine fetal death (p = 0.155). There was no significant difference in the history of intrauterine growth restriction between the subjects and the control group (1.9 versus 3.7%; p = 0.560).

Table 2: Comparison of gynecological history between subjects and controls

Variables	Control group n (%)	Subjects n (%)	χ^2	p-value
No of pregnancies				
0	17 (22.7)	0 (0.0)	21.064	*<0.0001
1	0 (0.0)	22 (29.3)		
2 – 3	44 (58.7)	42 (56)		
> 3	14 (18.7)	11 (14.7)		
Previous miscarriage				
Yes	20 (34.5)	20 (37.7)		0.873
No	38(65.5)	33 (62.3)		
Number of miscarriages				
One	14 (70.0)	15 (75.0)		0.915
Two	4 (20.0)	3 (15.0)		
Three	4 (10.0)	2 (10.0)		
High blood pressure in pregnancy				
Yes	4 (6.9)	4 (5.3)		0.993
No	54 (93.1)	71 (94.7)		
Need for cervical cerclage				
Yes	0 (0.0)	3 (5.7)		0.341
No	58 (100)	50 (94.3)		
Intrauterine foetal death				
Yes	0 (0.0)	2 (3.8)		0.436
No	58 (100)	51 (96.2)		
Delivery before 37 weeks	6 (10.3)	10 (18.9)		0.314
Yes	52(89.7)	43 (81.1)		
No				

Intrauterine growth restriction	2 (3.7)	1 (1.9)		1.000
Yes	56 (96.6)	52 (98.1)		
No				

χ^2: Chi square statistic, p-value < 0.05 indicates significance

Table 3 depicts that there was no significant difference in family history of deep venous thrombosis, pulmonary embolism, bleeding disorder and sudden death during pregnancy between the two groups (p-value > 0.05).

Table 3: Comparison of family history of thromboembolic/ bleeding disorders between subjects and control group

Variables	Control group n (%)	Subjects n (%)	χ^2	p-value
History of deep venous thrombosis				
Yes	2 (2.7)	0 (0.0)	2.027	0.155
No	73 (97.3)	75 (100)		
History of pulmonary embolism				
Yes	0 (0.0)	0 (0.0)	NA	NA
No	75 (100)	75 (100)		
Bleeding disorder				
Yes	0 (0.0)	2 (2.7)	2.027	0.155
No	75 (100)	73 (97.3)		
Sudden death during pregnancy	0 (0.0)	1 (1.3)	1.007	0.316
Yes	75 (100)	74 (98.7)		
No				

*χ^2: Chi square statistic, *p-value < 0.05 indicates significance, NA: Statistics not computed*

Table 4; there was no significant difference in the mean protein C between the subjects in the first trimester and the control group, p-value = 0.4890. Antithrombin III was significantly higher among subjects in the first trimester than the controls (603.52 ± 56.43 ng/mL and 554.16 ± 54.65 ng/mL respectively), p-value = 0.0004. Similarly, Protein S was significantly higher among subjects in the first trimester than the controls (5.77 ± 0.69 ng/mL and 4.78 ± 0.65 ng/mL respectively), p-value < 0.0001. However, Plasma level of natural coagulation inhibitors among subjects in the second trimester did not differ significantly from controls. Antithrombin III was

significantly lower among subjects in the third trimester than the controls (530.00 ± 48.13 ng/mL and 554.16 ± 54.65 ng/mL respectively), p-value = 0.0413. There was also a downward trend in the natural coagulation inhibitors as pregnancy advanced in this study.

Table 4: Comparison of the plasma level of natural coagulation inhibitors among subjects in the subjects and controls

Gestational period	Variables	Control group	Subjects	t-value	p-value
First trimester	Protein C (pg/mL)	1051.80 ± 240.04	1094.08 ± 244.04	-0.683	0.4890
	Antithrombin(III) (ng/mL)	554.16 ± 54.65	603.52 ± 56.43	-3.817	*0.0004
	Protein S (ng/mL)	4.78 ± 0.65	5.77 ± 0.69	-6.280	*<0.0001
Second trimester	Protein C (pg/mL)	1051.80 ± 240.04	1064.00 ± 241.18	-0.219	0.8275
	Antithrombin(III) (ng/mL)	554.16 ± 54.65	570.76 ± 57.63	-1.263	0.2140
	Protein S (ng/mL)	4.78 ± 0.65	4.84 ± 0.75	-0.372	0.7110
Third trimester	Protein C (pg/mL)	1051.80 ± 240.04	1043.80 ± 218.04	0.154	0.8770
	Antithrombin(III) (ng/mL)	554.16 ± 54.65	530.00 ± 48.13	2.099	*0.0413
	Protein S (ng/mL)	4.78 ± 0.65	4.84 ± 0.73	-0.397	0.6935

t: t-statistic, p-value < 0.05 indicates significance

Table 5 shows that Protein C, Protein S and antithrombin III all declined with gestational age. The mean protein C decreased from the first to the third trimester (first: 1094.1 ± 276.7pg/ml, second: 1064 ± 241.2pg/ml, third: 1043.8 ± 218.0pg/ml). These differences were not significant (p=0.769). The mean protein S among subjects in the first trimester was 5.7 ± 0.7ng/ml and 4.8 ± 0.7ng/ml in both the second and third trimesters. These differences were statistically significant (p=0.001). The mean antithrombin III decreased from the first trimester to the third trimester (first: 603.5 ± 56.4ng/ml, second: 570.8 ± 57.6ng/ml, third: 530.0 ± 48.1ng/ml). These differences were statistically significant (p=0.001).

Table 5: Comparison of natural plasma coagulation inhibitors among pregnant women in different gestation periods

Variables	Gestation period (Trimester)			F	p-value
	1st	2nd	3rd		
Protein C (pg/mL)	1094.1 ±	1064.0 ± 241.2	1043.8 ±	0.263	0.769
Protein S (ng/mL)	276.7	4.8 ± 0.7	218.0	13.623	*0.001
Antithrombin	5.7 ± 0.7	570.8 ± 57.6	4.8 ± 0.7		
III (ng/mL)	603.5 ± 56.4			11.533	*0.001
			530.0 ± 48.1		

***F: F statistic, p-value < 0.05 indicates significance*

Discussion

The mean age of the pregnant women was 32.6 ± 4.6 years while the mean age of participants in the control group was 34.5 ± 6.9 years and the majority of the participants were aged between 30-34 years. This finding is similar to the age range found in the work done by Oladosu *et al* who reported a mean age of 29.9 ± 5.2 years.[17] Okwesili *et al* however reported a lower mean age (25.52 ± 4.92 years) in a study done in Sokoto Nigeria, this may be a result of the practice of early marriage among women in the northern part of Nigeria. Age is an important factor in the likelihood of occurrence of thrombophilia and pregnancy complications; the older age group is associated with higher risk.

Most of the participants (70.7%) in this study were Christians which reflected the dominance of Christians in Ogbomoso. 98.7% of the participants in the study group were married while 84.0% in the control group were married. This finding is similar to a study done by Okeyinka Y. who reported that 78.2% of women in Ogbomoso were married.[18]

As expected, the dominant ethnic group of participants (Study group-93.3%, Control group 100%) was Yoruba since this study was conducted in the South Western part of Nigeria, a Yoruba-dominated population. This study revealed that the majority of the participants in both groups (94.7%-Control group, 85.3%-study group) had a tertiary level of education. This may reflect the fact that more highly educated people patronized the hospital being a teaching hospital than non-educated individuals.

The menstrual cycles of all the subjects and controls were within the normal range and likewise, the duration of bleeding for subjects and controls was within the normal range (100%). This might be because both the subjects and control group were apparently normal without any morbidity as stated in the inclusion criteria. Approximately 38% and 35% of the study group and control respectively had a history of miscarriages.

The plasma level of Protein C, Protein S, and Antithrombin III for both groups were within the normal reference range but slightly higher among the pregnant women than the control subjects. However, these differences were not statistically significant. This study revealed that there is just a slight increase of the NCI in normal pregnancy to combat the hypercoagulable state of pregnancy thereby preventing the occurrence of thromboembolic events associated with pregnancy. There was also a downward trend of the NCI as the gestation period increased which corroborated that pregnancy is a hypercoagulable state with the likelihood of consumption of NCI as pregnancy advanced. A study done in Turkey by Cengiz *et al* had a similar report.[12] Also, Nwagha *et al* reported a decrease in protein C from the second to the third trimester.[7]

However, Imoru *et al* have a contradictory report with protein C increasing significantly across the trimesters while antithrombin III fluctuated across the trimesters when compared with control.[3] This might be because of the small sample size that was studied (n=10). It was also

noted in a study done by Buseri *et al* that protein C was significantly lower when compared with the control while antithrombin III showed no significant difference however the study group was not divided into trimesters, which might account for the contrary opinion.[19]

The mean antithrombin III and protein C of the pregnant women decreased across the gestation period from the first trimester to the third trimester with statistical significance only in antithrombin III (p=0.001). This is in keeping with the hypercoagulable state of pregnancy and increased consumption of the NCI as the pregnancy progressed to the third trimester.[2,5,6] Nwagha *et al* reported the same pattern with protein C.[7] Other reports by Imoru *et al* and Ajayi *et al* showed different patterns and fluctuated values for protein C and antithrombin III at different gestation periods with no statistical significance.[3,19]

The mean protein S of the normal pregnant women decreased significantly from the first trimester to the second trimester and maintained at that level throughout the pregnancy. Domenico *et al* and Feroza *et al* reported a progressive fall of total protein S with increasing gestation age.[11,20] Variation of protein S by various authors during pregnancy could be associated with different types of reagents used, techniques employed and sample sizes considered.

Conclusion

Pregnancy is a hypercoagulable state regulated by natural coagulation inhibitors (NCI) which were higher among subjects in this study and were also noted to decrease as pregnancy advanced. It is therefore recommended that all pregnant women with high-risk factors of thromboembolic events should have a plasma level of Prothrombin Time, and Activated Partial Thromboplastin Time done as baseline investigations to identify the disorders early enough. Such pregnant women with deranged coagulation profiles could benefit from anticoagulation either prophylaxis or therapeutic and further clinical evaluations.

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